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IBOGAINE ANTAGONIZES COCAINE-INDUCED LOCOMOTOR STIMULATION IN MICE

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(Received in final form January 30, 1992)

Summary

Ibogaine (40 mg/kg i.p.), when given 2 hours before an acute injection of cocaine (25 mg/kg s.c.) to C57BL/6 mice, reduced the cocaine-induced locomotor stimulation. Such stimulation was also reduced in the ibogaine-treated mice when a second injection of cocaine was given 24 hr later. Thus, the reduction in locomotor activity was not just the short-term depression of locomotor activity seen after ibogaine administration. When mice were given a daily injection of cocaine for 3 days and ibogaine was given after the cocaine injection on day 3, and again on day 4, cocaine-induced locomotor activity was reduced three hours later on day 4. On days 5 and 9 of the cocaine administration, with no further ibogaine treatment ambulatory counts were still lower in the ibogaine-pretreated mice. Locomotor stimulation induced by amphetamine (10 mg/kg) was not affected by ibogaine. An acute injection of ibogaine resulted in a transient increase in turnover of dopamine, as indicated by the increase in the ratio of metabolites of the dopamine to dopamine, followed by a decrease in the metabolites in striatum and frontal cortex 24 hr later. In vivo treatment with ibogaine did not affect the binding of [³H]WIN 35,248 to the cocaine binding site in striatal tissue measured in vitro. In addition, ibogaine added in vitro had a weak affinity to the WIN 35,248 binding site (IC₅₀ for cocaine = 120 nM and for ibogaine = 1,500 nM). The results suggest that ibogaine may have induced a selective change in the dopaminergic system that results in a decrease in responsiveness to cocaine that persisted for at least 1 week.

Ibogaine (NIH 10567, EndabuseTM), the principal alkaloid of *Tabernaemontana iboga* Baillon, has recently been suggested to interrupt the physiological and psychological aspects of the opiate

withdrawal syndrome in humans (patent No. 4,499,096) and cocaine and amphetamine abuse syndromes (patent No. 4,587,243). However, these reports are only suggestive, and more compelling evidence is needed to confirm that ibogaine exhibits properties consistent with claims about its behavioral or pharmacological effects.

Maisonneuve et al. (1) have shown that repeated injections of ibogaine (40 mg/kg) decreased i.v. morphine self-administration by rats for several days. In addition, the rise in extracellular dopamine, measured with microdialysis techniques, as a response to morphine administration was prevented (1). In chronic morphine-dependent rats administration of naloxone induced a withdrawal syndrome, i.e., increased rearing, digging jumping, and salivation. Ibogaine significantly dose-dependently reduced the frequency of these withdrawal symptoms (2). In addition, all animals pretreated with ibogaine demonstrated decreased locomotion during withdrawal. Ibogaine decreased motor activity in addictive animals during a naloxone-precipitated withdrawal syndrome. Recently, Glick et al. (3) reported that ibogaine pretreatment inhibited intravenous self-administration of morphine in rats.

The locomotor stimulatory effect of cocaine is mediated by blockade of the uptake of dopamine. Since ibogaine may interact with the dopaminergic system, the present experiments examined the effect of ibogaine on cocaine-induced locomotor stimulation in mice.

Methods

Animals and Drugs: C57BL/6 adult male mice (4-6 months old) were used. Ibogaine HCl (Sigma Chemical Co.) (40 mg/kg i.p.) was dissolved in saline with warming. Cocaine hydrochloride (25 mg/kg s.c.) or amphetamine (10 mg/kg, s.c.) was dissolved in saline and injected at the indicated times.

Activity Apparatus: Locomotor activity was measured with an infrared beam-based activity sensor (Opto-Varimex-3, Columbus Instruments). Each mouse was placed in an individual cage (27 X 17 X 12 cm) one day before administration of drugs and were kept in the home cage throughout the experiment, with cage cleaning done the night before activity measurements. Ibogaine and cocaine were administered at times indicated in legends to figures. Animals were placed back in their home cages after drug or saline administration. Total ambulatory counts (TAC) indicates the total number of beams, on both the X and the Y axis, that have been interrupted by the animal during the ambulatory activity. Single beams broken repeatedly by the animals scratching or other stereotypic activity are not counted (Columbus Inst. Auto-Track System). TAC were calculated as the average of three 10-min periods from 30 to 60 min after injection of the drug. Saline and ibogaine-pretreated animals were tested concurrently.

Amine Determination: Mice were administered ibogaine (40 mg/kg) and killed at the times indicated. Amines and metabolites were assayed by HPLC and electrochemical detection in tissue extracts as described previously (4).

[³H]WIN 35,248 Binding: [³H]WIN 35,248 was used to label the cocaine binding site in striatal tissue homogenates as described by Boja et al. (5). Mice were injected twice with ibogaine (40 mg/kg), 6 hours apart, and killed the following day. Tissue homogenates prepared in a phosphate-sucrose buffer were incubated in the presence of 0.5 nM [³H]WIN 35,248. Non-specific binding was defined in the presence of 30 μ M cocaine.

In vitro competition studies for the [³H]WIN 35,248 binding site were also done, by adding increasing amounts of ibogaine to striatal tissue preparations containing 0.5 nM labeled WIN 35,248.

Statistical Analyses: Data were analyzed by using analysis of variance (ANOVA) and a post-hoc protected *t*-test (GB-STAT program)

Results

An acute injection of ibogaine (40 mg/kg i.p.) reduced general locomotor activity measured 1 hour after injection (Fig 1). Two and 24 hr after ibogaine, cocaine (25 mg/kg s.c.)-induced locomotor stimulation was significantly reduced (Fig. 1). Twenty-four hr after ibogaine, locomotor activity after a saline injection (prior to the cocaine injection) was not significantly different. Amphetamine (10 mg/kg s.c.)-induced locomotor activity measured 2 hours after ibogaine was not reduced (Fig 1).

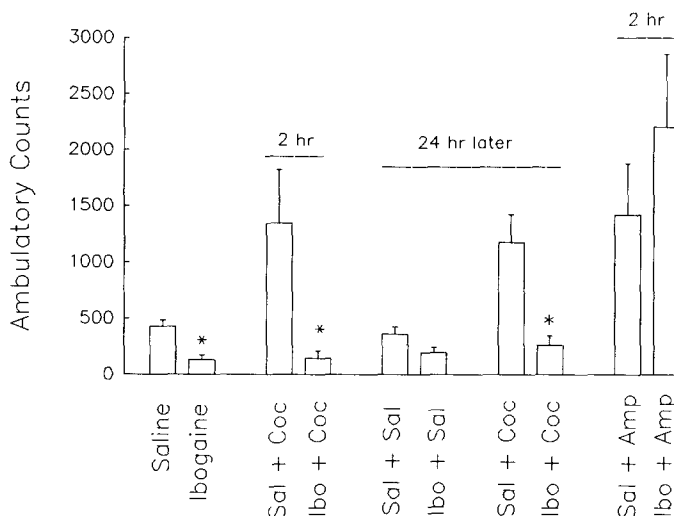


FIG. 1

The effect of ibogaine (40 mg/kg) on locomotor activity induced by cocaine (25 mg/kg) or amphetamine (10 mg/kg) in C57BL/6 male mice. Stimulant drugs were administered two hours after ibogaine. Cocaine was administered again 24 hr later. Results are the average ambulatory counts of three 10-min periods, from 30 to 60 min after cocaine or amphetamine administration (**p* < 0.01, *n* = 4-8).

Repeated cocaine injections increased the TAC (average of three 10-min periods from 30 to 60 min after cocaine) on days 1-3 (Fig 2). Ibogaine (40 mg/kg) was administered on day 3 after cocaine, and again on the morning of day 4. On day 4, three hours after the ibogaine, cocaine was administered. The TAC after cocaine was significantly lower at the 1 hour point in the ibogaine pretreated mice (Fig 2).

Without further ibogaine administration, cocaine was again administered on days 5 and 9 (Fig 3). The mice were first challenged with saline, followed by cocaine. In the ibogaine-pretreated mice, cocaine-induced locomotor activity was still significantly lower when measured on day 5 and day 9 (5 days after the last injection of ibogaine) (Fig. 3).

In striatum (Table 1), DOPAC and HVA levels increased 30-60 min after ibogaine injection. By 24 hours, the level of HVA remained lower than control values in striatum (Table 1) and in the frontal cortex, olfactory tubercle, and hippocampus (not shown). The level of dopamine decreased at 120 min after ibogaine, but returned to control value at 24 hr. There was a transient increase in the ratio of metabolites of dopamine (DOPAC + HVA) to dopamine after injection of ibogaine. The serotonin

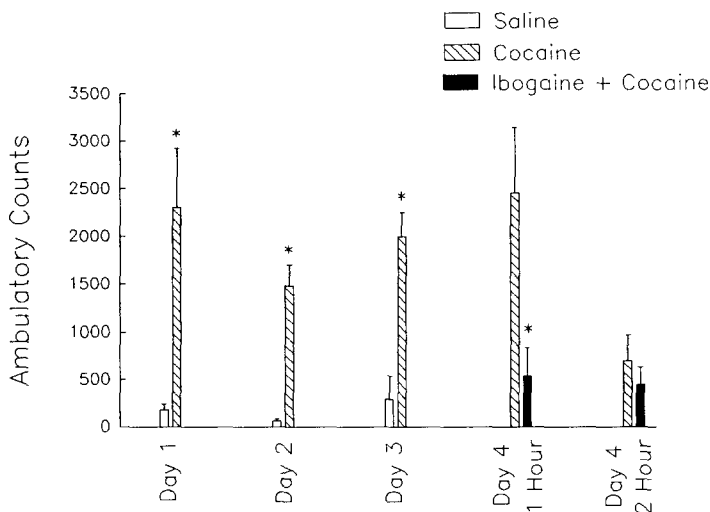


FIG. 2.

The effect of repeated cocaine injections (25 mg/kg daily for 3 days) on ambulatory counts. On day 3, three hours after the cocaine injection, half the group of animals were injected with ibogaine. On day 4, the animals were injected once more with ibogaine. Three hours later, cocaine was administered and activity was measured over a 2 hr period (results expressed as the average activity counts of three 10-min periods taken at 30-60 and 90-120 min after cocaine). (* $p < 0.01$, $n = 8$).

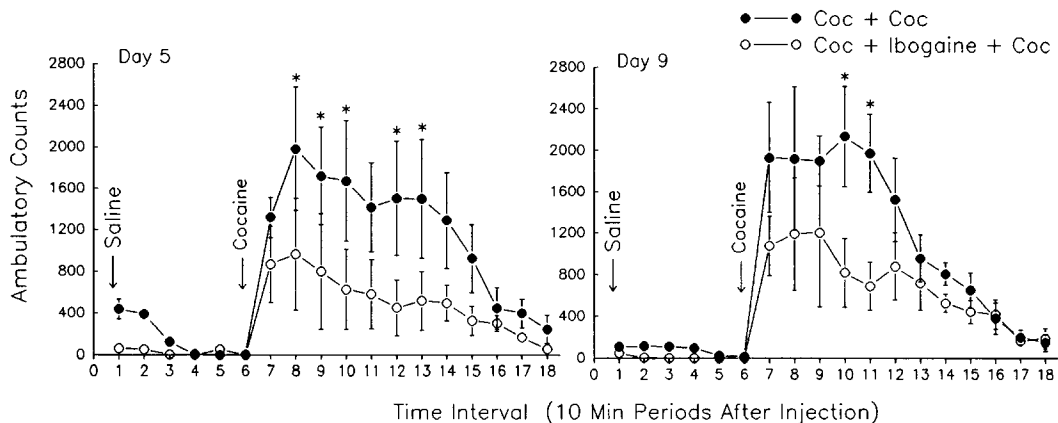


FIG. 3.

The effect of ibogaine administration (injections as above, on day 3 and 4) on cocaine-induced ambulatory counts measured on days 5 and 9. Both groups of animals were first injected with saline, and activity was measured for 1 hr (in 10-min segments), followed by an injection of cocaine, and activity was measured for another 2 hr (in 10-min segments). (* $p < 0.05$, $n = 8$).

metabolite 5-HIAA was lower at 24 hr after ibogaine in frontal cortex (Table 1) and hippocampus and olfactory tubercle (not shown).

TABLE 1.

Levels of Biogenic Amines and Metabolites After Acute Ibogaine

Region	Time after Ibogaine (pmol/mg protein)				
	Control	30 min	60 min	120 min	24 hr
Striatum					
DA	686±79	529±41	422±40*	355±20**	662±22
DOPAC	41±6	102±6**	81±9**	50±3	49±3
HVA	134±8	162±10	198±28**	165±2	99±4*
DOPAC+HVA	0.27	0.51	0.66	0.60	0.29
DA	±0.01	±0.03	±0.04	±0.04	±0.05
Frontal Cortex					
5HT	28±1	23±1**	26±2	25±2	26±1
5-HIAA	9.5±1.2	9.3±0.4	10.0±0.9	7.8±0.4**	7.5±0.7**

Ibogaine (40 mg/kg) was given and animals were killed at times indicated and amines and metabolites were determined in tissue extracts by electrochemical detection (* $p < 0.05$, ** $p < 0.01$, $n = 6$).

Ibogaine administered in vivo did not affect the binding of [3 H]WIN 35,248 to striatal tissue when measured the day after two injections of ibogaine. Binding activities in the presence of 0.5 nM [3 H]WIN 35,248 were $63,572 \pm 2,813$ and $66,334 \pm 2,892$ dpm/mg protein for control and ibogaine-treated mice ($n=6$).

Ibogaine added in vitro showed only a weak affinity for the [3 H]WIN 35,248 binding site. The IC_{50} value of ibogaine to the WIN 35,248 site was approximately 10 times higher than for cocaine (IC_{50} for cocaine = 120 nM and for ibogaine = 1,500 nM).

Discussion

The present study was undertaken to determine whether ibogaine antagonized the locomotor stimulation induced by cocaine in mice, and whether this effect was long-lasting. It is generally accepted that inhibition of dopamine reuptake underlies the locomotor stimulation induced by cocaine. It has been recently reported that with ibogaine pretreatment in rats, the cocaine-induced dopamine release measured in nucleus accumbens by in vivo voltammetry was inhibited without dopamine depletion, and cocaine-induced hyperactivity was also inhibited (6, P.A. Broderick, personal communication). However, Maisonneuve et al. (7) found that pretreatment with ibogaine did not modify the increase in dopamine induced by cocaine, but enhanced the rise in dopamine by amphetamine in the nucleus accumbens measured by microdialysis, and furthermore increased both amphetamine and cocaine-induced motor activity in rats. Ibogaine also prevented the rise in dopamine to a morphine challenge (8), and decreased morphine self-administration (3).

The present results show that ibogaine (at the same dosage as reported in other studies) decreases the locomotor response induced by cocaine. Although ibogaine appears to depress locomotor activity acutely, the effect is unlikely to be long-term. Activity measurements during the night, when the normal locomotor activity increases, appeared to be normal in the ibogaine-treated mice (TAC for 15 hours during the night were $27,651 \pm 3,772$ and $23,106 \pm 5,242$ counts for control and ibogaine mice, $n=4$). In addition, there was no apparent effect of ibogaine on general locomotor activity measured 1 (Figure 1) and 5 (Figure 3) days later, long after the administration of ibogaine. In rats trained to bar-press for water, ibogaine (40 mg/kg) acutely depressed the response; however, there was no evidence of any after-effects a day or more later (3).

Further experiments are needed to establish whether the effect of ibogaine is mediated via alteration of the cocaine binding site or via other metabolic effects. We saw no evidence of changes in the properties of [3 H]WIN 35,248 binding to the dopamine transporter. In addition, ibogaine has a weak affinity to this binding site compared to cocaine. Metaphit, an analog of phencyclidine (PCP), inactivates PCP receptors by acylation (9). Metaphit also prevents the locomotor activation induced by cocaine, but not amphetamine. Similarly, we found no evidence that it affects uptake sites for dopamine (9). We had suggested that an increased rate of dopamine catabolism in metaphit-treated

mice is responsible for the inhibition of locomotor stimulation (10). The increase in the ratio of metabolites of dopamine to dopamine suggest that ibogaine transiently enhances dopamine turnover and catabolism.

The serotonin metabolite 5-HIAA was also lower in frontal cortex, hippocampus, and striatum after 24 hr. The basis for these changes is not understood; however, effects of ibogaine on the serotonin system could be relevant to its action, possibly through modulation of dopamine release. Presynaptic serotonin sites can modulate dopamine release; for example, serotonergic innervation of the anterior striatum may exert a facilitatory influence on dopamine release (12). Long and Lerrin (13) have shown that ibogaine is a reversible inhibitor of the active transport of serotonin into blood platelets, as are cocaine and amphetamine. The cocaine binding site has also been associated with the dopamine and serotonin reuptake carrier (14), suggesting that ibogaine may interact with both these sites in the mediation of its effects. It is also possible that ibogaine acts by a lesioning effect, perhaps one that is brain region specific; however, longer term studies and repeated injections of ibogaine would be necessary to confirm such a mechanism..

Glick et al. (3) suggested the possibility of an active metabolite of ibogaine with a long half-life as an underlying mechanism of action. The selective effect of ibogaine on cocaine-versus amphetamine-induced locomotor activity further substantiates the different sites of action of these two stimulants (11).

The above results are not in conflict with the proposed uses of ibogaine in the treatment of cocaine abuse, since increased dopamine neurotransmission has been shown to be associated with the locomotor-stimulant and reinforcing effects of cocaine. Attenuation of these effects by ibogaine could possibly reduce the craving for cocaine. Further studies are needed on its neurochemical mechanism of action for an understanding of its potential usefulness in the treatment of drug abuse.

Acknowledgements

The authors thank Eric Chenven, a summer student from Brandeis University, for his help with the radioligand binding assays, and Mr. Howard Lotsof (NDA International, Inc.) for his discussions on ibogaine.

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